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DATA EVALUATION RECORD

STUDY TYPE: Repeated Dose 28-Day Oral Toxicity Study in Rats; OPPTS 870.3050; OECD 407.

PC CODE: 088001 **DP BARCODE:** 369393
TEST MATERIAL (PURITY): Copper pyrithione (99.7% a.i.)

SYNONYMS: Copper-2-pyrithio-1-oxide, Copper Omadine

CITATION: Omori, M. (1995) A repeated dose toxicity study of copper pyrithione administered orally to rats for 28 days followed by a 14-day recovery period. Shin Nippon Biomedical Laboratories, Ltd, Kagoshima, Japan. Laboratory Project ID: SBL 40-46, July 18, 1995. MRID 45774311. Unpublished.

SPONSOR/SUBMITTER: Arch Chemicals Inc., 501 Merritt 7, Norwalk, CT

EXECUTIVE SUMMARY: In a repeated dose, oral toxicity study (MRID 45774311), copper pyrithione (99.7% a.i.; Batch No. 9302095981) in 0.5% (w/v) carboxymethylcellulose was administered daily via gavage (10 mL/kg) to Sprague-Dawley rats at dose levels of 0, 0.6, 2.5, or 10 mg/kg/day for 28 days; the control and high-dose groups used 10 rats/sex/group and the low and mid-doses used 5 rats/sex/group. For the 0 and 10 mg/kg/day groups, 5 rats/sex/group were retained for a 14-day recovery period.

At 10 mg/kg/day, two females were sacrificed *in extremis* on Day 17 of the dosing period. Additionally, one 10 mg/kg/day female was found dead on Day 2 of the recovery period. These three animals exhibited the following clinical signs prior to death or euthanasia: emaciation; decreased spontaneous activity, piloerection, ataxic gait, paralysis of the hind leg, urine-stained abdomen, reddish eye gum; prone position; and lateral position. Additionally, hypothermia and bradypnea were noted in the females sacrificed in moribund condition, and a trace of reddish rhinorrhea was observed in the animal found dead. Among the surviving 10 mg/kg/day females, similar clinical signs of emaciation, piloerection, decreased spontaneous activity, ataxic gait and/or paralysis of the hind leg; reddish eye gum; urine-stained abdomen; and prone position were found. However, almost all of these findings decreased in severity or occurrence during the final week of dosing. During the initial recovery period, decreased spontaneous activity, ataxic gait, and piloerection were still observed in 1 or 2 females, but these abnormalities disappeared by Day 5 of the recovery period. Emaciation was also observed in 2 females during the recovery period, but these animals appeared to be recovering.

No treatment-related clinical signs were noted in the 10 mg/kg/day males, other than slight emaciation in three males on Days 21 to 28 or on Days 0-2 of the recovery period,

Body weights were decreased ($p<0.05$) by 10-13% in the 10 mg/kg/day males during Weeks 3 and 4 and by 19-36% in the females during Weeks 2-4. During the recovery period, body weights remained decreased by 13% in these males during Week 1 and by 25-33% in the females during Weeks 1 and 2. Body weight gains were decreased ($p<0.05$) by 18-39% in the males during Weeks 2-4. The females at this dose actually lost significant ($p<0.01$) weight during Week 2 (-0.6 g) and Week 3 (-18.8 g) compared to gains of 24.6 and 28.6 g, respectively, in the controls. Overall (Weeks 0-4) body weight gain was decreased by 22% in the males and 76% in the females. During the recovery period, body weight gains were increased compared to controls by 28% in the males during Week 2 and by 82-123% in females during Weeks 1 and 2. Food consumption was decreased ($p<0.01$) by 11-12% in the males during Weeks 3 and 4 and by 34-37% in the females during Weeks 2 and 3. Food consumption was similar to controls in both sexes during the recovery period.

At the high-dose, treatment-related gross lesions were limited to slight to moderate atrophy of the biceps femoris in 2/5 females and slight to marked emaciation in all females at the end of the dosing period. Microscopic observations of muscle fiber atrophy were observed in the gastrocnemius, soleus, flexor digitorum longus, and anterior tibial muscles in all (5/5) females at this dose compared to 0/5 controls. Additionally, muscle fiber atrophy of the biceps femoris was found in 2/5 females at 10 mg/kg/day compared to 0/5 controls. In the high-dose males, muscle fiber atrophy in the anterior tibial muscle was observed in 2/5 rats compared to 0/5 controls. The severity of the muscle fiber atrophy was generally very slight to slight.

No treatment-related effects were observed at 0.6 or 2.5 mg/kg/day in either sex.

The LOAEL was 10 mg/kg/day based on decreased body weight, body weight gain, and food consumption in both sexes and on mortality, clinical signs of toxicity, and muscle fiber atrophy in the females. The NOAEL is 2.5 mg/kg/day.

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3050; OECD 407) for a subchronic oral toxicity study in the rat.

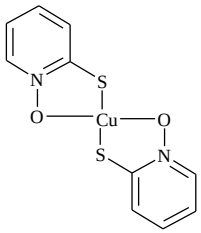
COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Copper pyrithione

Description: Olive-green powder
Batch #: 9302095981
Purity: 99.7% a.i.
Stability: The test material was shown to be stable in the vehicle for up to 24 hrs at room temperature.
CAS # of TGAI: 14915-37-8
Structure:



2. Vehicle: Aqueous 0.5% (w/v) carboxymethylcellulose

3. Test animals

Species: Rat
Strain: Sprague-Dawley, SPF Crj:CD(SD)
Age/ weight at study initiation: Approximately 5 weeks/ 143-161 g males and 121-134 g females
Source: Charles River Japan, Inc.
Housing: Individually in stainless steel cages
Diet: CE-2 solid food (sterilized by cobalt-60 irradiation ; CLEA Japan, Inc.), *ad libitum*; except during fasting prior to blood collection
Water: Tap water, *ad libitum*
Environmental conditions
Temperature: 22±2°C
Humidity: 50±10%
Air changes: 15/hr
Photoperiod: 12 hrs dark/ 12 hrs light
Acclimation period: 8 days

B. STUDY DESIGN

1. In-life dates: Not reported

2. Animal assignment: The animals were randomly assigned, stratified by weight, to the test groups presented in Table 1.

TABLE 1. Study design [a]

Test group	Dose (mg/kg/day)	# Males [b]	# Females [b]
Control	0	5+5	5+5
Low	0.6	5	5
Mid	2.5	5	5
High	10	5+5	5+5

[a] Data were extracted from page 15 of the study report.

[b] An additional 5 rats/sex were added to the control and high-dose groups for the recovery test.

3. **Dose-selection rationale:** The doses in the current study were based on the results of a preliminary repeated dose toxicity study (Report No. SBL 40-45; Omori, M., 1995), in which rats were exposed orally to copper pyrithione at 1, 5, and 25 mg/kg/day for 2 weeks. It was determined that 25 mg/kg/day caused significant toxicity but no mortality, and 5 mg/kg/day caused no significant toxicity. Therefore, 10 mg/kg/day was selected as the high-dose in the current study.

4. **Treatment preparation, administration, and analysis:** Dose formulations were prepared daily by mixing the appropriate amounts of copper pyrithione and 0.5% (w/v) carboxymethylcellulose. The animals were dosed daily via gavage at a volume of 10 mL/kg based on the most recent body weight. Stability after 24 hrs at room temperature and homogeneity (top, middle, bottom) were evaluated in 0.06, 0.10, and 2.5 mg/mL formulations prior to the study. Concentration analyses were performed using samples from each dose formulation on the initial day of dosing and at Week 4 during the current study.

Results

Homogeneity analysis (%CV): 0-1%

Stability analysis (% of initial value): 94.9-101.7% (after 24 hrs at room temperature)

Concentration analysis (range as mean % of nominal):

Concentration (mg/mL)	Mean % of nominal
0.06	95-100
0.25	97.6-104
1.0	100-108

The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was acceptable.

5. **Statistics:** The following statistical methods were applied to the data, and significance was denoted at $p<0.05$ or $p<0.01$. Body weight, food consumption, hematology, clinical chemistry, and organ weight data were first analyzed for homogeneity of variance by Bartlett's test. If homogeneity of variance existed, a one-way ANOVA was applied. If the ANOVA was significant, Dunnett's test (equal numbers of data) or Scheffé's test (unequal

number of data) was used to compare the treatment and control groups. If the variance was heterogeneous in Bartlett's test, the order was converted and the Kruskal-Wallis H test was applied. If the results were significant, Dunnett-type test (equal numbers of data) or Scheffe-type test (unequal numbers of data) was used to compare the mean ranks. Urinalysis, macroscopic pathology, and microscopic pathology data were analyzed using either the exact rank sum test (gradable values) or the Fisher's exact test (non-gradable values). Clinical signs data were not subjected to statistical analysis.

C. METHODS

1. Observations

a. Cageside observations: Animals were observed for mortality and clinical signs of toxicity three times daily (prior to dosing, and at approximately 1-2 hr and 4-5 hr post-dosing) during the dosing period, and once daily during the recovery period.

b. Clinical examinations: It was not reported if the animals were subjected to detailed physical examinations.

c. Neurological evaluations: All animals were observed for general behavior according to the Irwin method[1] (a modified FOB) once prior to initiation of dosing and on Days 0, 6, 13, 20, and 27 of the dosing period and on Day 6 and 13 of the recovery period.

The parameters examined included, but were not limited to, the following: awareness (alertness, visual placing, stereotypy, and passivity); mood (grooming and vocalization); motor activity (reactivity, spontaneous activity, touch response, and pain response); central nervous system excitation (startle response, straub tail, tremors, convulsions, and twitches); posture; motor incoordination (staggering gait, abnormal gait, and righting reflex); muscle tone (grip strength, body tone, and abnormal tone); reflex (pinna reflex, corneal reflex, and ipsilateral flexor reflex); and autonomic profile (writhing, palpebral opening, exophthlmos, urination, salivation, piloerection, hypothermia, skin color, heart rate, and respiratory rate. Details of the scoring scale were not provided in the study report.

2. Body weight: All animals were weighed once prior to initiation of dosing and weekly thereafter. Body weight gains were also calculated weekly.

3. Food consumption: Food consumption (g/animal/day) was calculated once prior to initiation of dosing and weekly thereafter.

4. Ophthalmoscopic examination: Ophthalmoscopic examinations were not performed.

1 Irwin S., Animal and clinical pharmacological techniques in drug evaluation. Ed. By Nodine, J.H. and Siegler, P.E., Chicago, Year Book Medical Publishers, 36-54, 1964.

5. Hematology and clinical chemistry: On the day of scheduled sacrifice, all surviving animals (fasted for 16-24 hr) were anesthetized by injection of sodium pentobarbital. Blood samples for hematology parameters were collected from the abdominal vein and treated with EDTA-2K or 3.8% sodium citrate (clotting measurements). Afterward, blood samples for clinical chemistry evaluations were collected from the abdominal aorta. The following CHECKED (X) parameters were examined:

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*		Erythrocyte morphology
X	(Activated partial thromboplastin time)		
	(Kaolin-cephalin time)		
X	(Prothrombin time)		

* Recommended for 28-day oral rodent studies based on Guideline 870.3050

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total cholesterol*
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes)	X	Total bilirubin
X	Alkaline phosphatase (ALP)*	X	Total protein*
X	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
X	Lactic acid dehydrogenase (LDH)	X	Albumin/globulin ratio
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 28-day oral rodent studies based on Guideline 870.3050

6. <u>Urinalysis</u>: Urine was collected using the compulsory method once prior to initiation of dosing, once during Week 4 (prior to daily dosing) of the dosing period, and once during Week 2 of the recovery period. Color was judged visually and the following parameters were determined using Multistix® test paper (Miles-Sankyo Co., Inc.).

X	Appearance*	X	Glucose
	Volume*	X	Ketones
	Specific gravity/osmolality*	X	Bilirubin
X	pH*	X	Blood/blood cells*
	Sediment (microscopic)		Nitrate
X	Protein*	X	Urobilinogen

* Optional for 28-day oral rodent studies

7. <u>Sacrifice and pathology</u>: All animals sacrificed *in extremis* and at scheduled necropsy were anesthetized by injection of sodium pentobarbital and euthanized after blood collection. The following CHECKED (X) tissues were collected, and the (XX) organs were also weighed (paired organs were weighed separately and together).

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue		Aorta*	XX	Brain*+
	Salivary glands*	X	Heart*+	X	Peripheral nerve*
	Esophagus*		Bone marrow*	X	Spinal cord (3 levels)*
	Stomach*		Lymph nodes*	X	Pituitary*
	Duodenum*	XX	Spleen*+		Eyes (w/optic nerve)*
	Jejunum*		Thymus*+		**GLANDULAR**
	Ileum*			XX	Adrenal glands*+
	Cecum*		**UROGENITAL**		Lacrimal glands
	Colon*	XX	Kidneys*+	X	Parathyroids*
	Rectum*	X	Urinary bladder*	X	Thyroid*
XX	Liver*+	XX	Testes*+		**OTHER**
	Gall bladder (not rat)*		Epididymides*+		Bone (sternum and femur)
	Bile duct (rat)		Prostate*	X	Skeletal muscle
	Pancreas*		Seminal vesicles*		Skin*
	RESPIRATORY	XX	Ovaries*+	X	All gross lesions and masses*
	Trachea*		Uterus*+		
XX	Lungs*		Mammary gland*		
	Nose*		Cervix		
	Pharynx*		Vagina		
	Larynx*				

* Recommended for 28-day oral rodent studies based on Guideline 870.3050
\+ Organ weights required for rodent studies.

The eyes were fixed in a mixture of formaldehyde and glutaraldehyde. The remaining collected tissues were fixed in 10% neutral buffered formalin. Tissues from 5 rats/sex/dose in the control and 10 mg/kg/day groups and the triceps muscle of the calf (gastrocnemius

muscle and soleus muscle), flexor digitorum longus muscle, anterior tibial muscle, liver, kidneys, and gross lesions from all groups were evaluated microscopically. Tissues were routinely processed, embedded in paraffin, stained with hematoxylin and eosin, and examined microscopically.

II. RESULTS

A. OBSERVATIONS

1. **Mortality:** At 10 mg/kg/day, two females (nos. 53 and 54) were sacrificed *in extremis* on Day 17 of the dosing period. Additionally, one 10 mg/kg/day female (no. 51) was found dead on Day 2 of the recovery period. All these deaths are treatment related.

2. **Clinical signs:** At 10 mg/kg/day, two females (nos. 53 and 54) were sacrificed in extremis on Day 17 of the dosing period. These animals displayed the following clinical signs: emaciation from Day 14 of the dosing period onward; decreased spontaneous activity, piloerection, ataxic gait, and/or paralysis of the hind leg, urine stained abdomen, and reddish eye gum on Day 15 and thereafter; prone position on Day 16; and hypothermia, bradypnea, and lateral position were noted on Day 17. While under observation using the Irwin method, no abnormalities were noted in either of these animals until Day 13 of the dosing period.

   Additionally, one 10 mg/kg/day female (no. 51) was found dead on Day 2 of the recovery period. This animal displayed the following clinical signs prior to death: emaciation from Day 17 of the dosing period onward; decreased spontaneous activity, ataxic gait and/or paralysis of the hind leg, urine stained abdomen, reddish eye gum, and a trace of reddish rhinorrhea from Day 18 onward; piloerection from Day 19 onward; prone position on Day 0 of the recovery period; and lateral position on Day 1 of the recovery period. While under observation using the Irwin method, an increase in passivity, decreases in reactivity, spontaneous activity, pain response, limb tone and grip strength, and loss of ipsilateral flexor reflex, abnormal hind limb posture and piloerection were observed on Day 20 and 27 of the dosing period. On Day 27, abnormal urination was also observed. All other animals survived to scheduled sacrifice.In the surviving 10 mg/kg/day females, emaciation was observed in all animals on Days 12 and 20 of the dosing period. Thereafter, piloerection decreased spontaneous activity, ataxic gait and/or paralysis of the hind leg were observed in 6 animals; reddish eye gum was noted in 3 animals; urine stained abdomen was noted in 2 animals; and prone position was noted in 1 animal. However, almost all of these findings disappeared or tended to recover by the end of the dosing period. Other than slight emaciation observed in three 10 mg/kg/day males on Days 21 to 28 or during Days 0-2 of the recovery period, no treatment-related clinical signs were noted in the males at any dose.

3. **Neurological evaluations (Irwin method):** No treatment-related differences from controls were noted in the males at any time-point or at any dose. Scores for all parameters were similar to controls prior to initiation of treatment and on Days 0, 6, and 13 of dosing in the

treated females. Incidence (# affected/8 vs. 0/10 controls) of the following observations were noted in the 10 mg/kg/day females on Day 20 (Table 2): (i) increased passivity (7); (ii) decreased reactivity (3); (iii) decreased spontaneous activity (5); (iv) decreased pain response (7); (v) abnormal hind limb posture (7); (vi) staggering gait (7); (vii) abnormal gait (5); (viii) decreased limb tone (7); (ix) decreased grip strength (7); (x) loss or decrease in ipsilateral flexor reflex (7); (xi) abnormal urination (2) and (xii) piloerection (7).. On Day 27, the same FOB parameters still showed an effect of treatment in the 10 mg/kg/day females; however, with the exception of reactivity and piloerection, the incidence and severity were generally less than that observed on Day 20. During the recovery period, a decrease in spontaneous activity and piloerection were observed in 2 females and ataxic gait was noted in 1 female continuously from the dosing period. These abnormalities disappeared by Day 5 of the recovery period. Emaciation was also observed in 2 females but appeared to be recovering.

TABLE 2. Incidence (# affected) of selected FOB parameters in female rats treated with copper pyrithione for up to 28 days. [a]

Observation	Score [b]	Dose (mg/kg/day)							
		0 (n=10)	0.6 (n=5)	2.5 (n=5)	10 (n=8)	0 (n=10)	0.6 (n=5)	2.5 (n=5)	10 (n=8)
		Day 20				Day 27			
Passivity (normal = 0)	0	10	5	5	1	10	5	5	5
	1	0	0	0	7	0	0	0	3
Reactivity (normal = 4)	4	10	5	5	5	10	5	5	4
	3	0	0	0	3	0	0	0	4
Spontaneous activity (normal = 4)	4	10	5	5	3	10	5	5	4
	3	0	0	0	0	0	0	0	2
	2	0	0	0	5	0	0	0	2
Pain response (normal = 4)	4	10	5	5	1	10	5	5	4
	3	0	0	0	2	0	0	0	2
	2	0	0	0	2	0	0	0	1
	1	0	0	0	3	0	0	0	1
Limb posture (normal = 4)	4	10	5	5	1	10	5	5	5
	3	0	0	0	7	0	0	0	3
Staggering gait (normal = 0)	0	10	5	5	1	10	5	5	5
	2	0	0	0	2	0	0	0	2
	-	0	0	0	5	0	0	0	1
Abnormal gait (normal = 0)	0	10	5	5	3	10	5	5	7
	-	0	0	0	5	0	0	0	1
Limb tone (normal = 4)	4	10	5	5	1	10	5	5	5
	3	0	0	0	5	0	0	0	2
	2	0	0	0	2	0	0	0	1
Grip strength (normal =4)	4	10	5	5	1	10	5	5	5
	3	0	0	0	7	0	0	0	3
Ipsilateral flexor reflex (normal = 4)	4	10	5	5	1	10	5	5	5
	3	0	0	0	2	0	0	0	0
	2	0	0	0	0	0	0	0	2
	0	0	0	0	5	0	0	0	1
Urination (normal = 0)	0	10	5	5	6	10	5	5	7
	1	0	0	0	1	0	0	0	1
	2	0	0	0	1	0	0	0	0
Piloerection (normal = 0)	0	10	5	5	1	10	5	5	0
	2	0	0	0	7	0	0	0	8

[a] Data were extracted from Tables 2-9 and 2-10 on pages 42 and 43 of the study report.

[b] Irwin method (1964)

B. <u>BODY WEIGHT AND BODY WEIGHT GAIN</u>: At 10 mg/kg/day, body weights were decreased ($p<0.05$) by 10-13% in the males during Weeks 3 and 4 and by 19-36% in the females during Weeks 2-4 (Table 3a). During the recovery period, body weights remained decreased by 13% in the males during Week 1 and by 25-33% in the females during Weeks 1-2. No effects on body weight were observed at 0.6 or 2.5 mg/kg/day in either sex.

TABLE 3a. Mean (±SD) body weights (g) in rats treated with copper pyrithione for up to 28 days. [a]

Interval (Weeks)	Dose (mg/kg/day)			
	0	0.6	2.5	10
Dosing period				
Males				
0	151.7±4.6	152.6±5.9	152.6±4.4	150.6±4.3
3	323.0±20.7	323.6±12.0	315.6±20.5	291.2±19.7* (↓10)
4	363.1±27.8	364.8±21.5	348.4±26.1	315.8±25.0** (↓13)
Females				
0	127.2±2.8	128.4±2.7	128.4±3.6	127.1±3.7
2	190.5±11.7	188.8±6.0	190.2±7.6	154.6±20.7** (↓19)
3	215.0±17.2	208.6±10.5	209.2±9.9	137.5±22.4** (↓36)
4	237.9±21.6	227.6±18.8	228.2±5.7	153.6±24.8** (↓35)
Recovery period				
Males				
1	398.6±38.5	--	--	347.0±31.3* (↓13)
2	428.2±41.1	--	--	384.8±33.5
Females				
1	245.4±23.4	--	--	164.5 (↓33)
2	258.2±21.4	--	--	193.0 (↓25)

[a] Data were extracted from Tables 4-1 and 4-2 on pages 46 and 47 of the study report. Percent difference from control is presented parenthetically.

* Significantly different from controls at $p<0.05$

** Significantly different from controls at $p<0.01$

Body weight gains were decreased ($p<0.05$) by 18-39% in the 10 mg/kg/day males during Weeks 2-4 (Table 3b). The females at this dose actually lost significant ($p<0.01$) weight during Week 2 (-0.6 g) and Week 3 (-18.8 g) compared to 24.6 and 28.6 g, respectively, in the controls. At 10 mg/kg/day, overall (Weeks 0-4) body weight gain was decreased by 22% in the males and 76% in the females. During the recovery period, body weight gains were increased compared to controls by 28% in the males during Week 2 and by 82 and 123% in females during Weeks 1 and 2, respectively.

TABLE 3b. Mean (±SD) body weight gains (g) in rats treated with copper pyrithione for up to 28 days. [a]				
Interval (Weeks)	Dose (mg/kg/day)			
	0	0.6	2.5	10
Dosing period				
Males				
1	59.0±7.1	60.8±5.6	58.4±7.8	55.8±5.8
2	62.0±6.1	62.0±3.3	58.8±4.0	51.0±8.8* (↓18)
4	40.1±10.9	41.2±10.3	32.8±9.2	24.6±8.0* (↓39)
Overall (0-4) gain [b]	211.4	212.2	195.8	165.2 (↓22)
Females				
1	34.7±7.8	32.6±3.6	35.8±4.1	28.1±5.7
2	28.6±4.6	27.8±5.5	26.0±3.6	-0.6±16.5**
3	24.5±7.2	19.8±6.3	19.0±2.7	-18.8±23.5**
4	22.9±6.2	19.0±9.1	19.0±5.5	16.1±16.5
Overall (0-4) gain [b]	110.7	99.2	99.8	26.5 (↓76)
Recovery period				
Males				
1	29.0±7.4	--	--	30.8±6.7
2	29.6±5.3	--	--	37.8±5.7* (↑28)
Females				
1	14.8±7.5	--	--	27.0 (↑82)
2	12.8±2.4	--	--	28.5 (↑123)

[a] Data were extracted from Tables 4-3 and 4-4 on pages 48 and 49 of the study report. Percent difference from control (calculated by reviewers) is presented parenthetically.

[b] Calculated by reviewers from data within Table 3a above.

* Significantly different from controls at $p<0.05$

** Significantly different from controls at $p<0.01$

C. <u>FOOD CONSUMPTION</u>: At 10 mg/kg/day, food consumption was decreased ($p<0.01$) by 11-12% in the males during Weeks 3 and 4 and by 34-37% in the females during Weeks 2 and 3 (Table 4). Food consumption was similar to controls in both sexes during the recovery period. No effects on food consumption were observed at 0.6 or 2.5 mg/kg/day in either sex.

TABLE 4. Mean (±SD) food consumption (g/animal/day) in rats treated with copper pyrithione for up to 28 days. [a]				
Interval (Weeks)	Dose (mg/kg/day)			
	0	0.6	2.5	10
Males				
0	21.6±1.3	21.6±1.3	21.4±1.3	20.9±1.2
3	28.2±2.5	28.6±1.7	26.8±1.9	24.7±1.8** (↓12)
4	28.7±2.2	27.4±3.5	24.8±2.6	24.3±2.5** (↓11)
Females				
0	18.7±0.7	17.8±1.6	19.0±1.9	18.5±1.6
2	20.0±1.8	19.6±1.5	20.0±2.0	13.2±4.4** (↓34)
3	20.8±1.7	19.2±1.3	19.6±2.3	13.1±5.6** (↓37)
4	20.8±3.0	19.0±2.7	19.8±1.5	16.6±6.1

[a] Data were extracted from Tables 3-1 and 3-2 on pages 44 and 45 of the study report. Percent difference from control (calculated by reviewers) is presented parenthetically.

** Significantly different from controls at $p<0.01$

D. OPHTHALMOSCOPIC EXAMINATION: Not performed.

E. BLOOD ANALYSES

1. **Hematology:** At 10 mg/kg/day, treatment-related differences ($p<0.05$) in hematology parameters were limited to decreased lymphocyte count in the males (↓31%) and increased segmented neutrophilic ratio in the females (6.8% treated vs. 2.8% controls). The increases in platelets (↑54%) and reticulocytes (↑50%) noted in the 10 mg/kg/day females at the end of the recovery period were considered incidental as these parameters were unaffected during dosing. All other statistically significant differences in hematology parameters were unrelated to dose.

2. **Clinical chemistry:** At 10 mg/kg/day, decreased ($p<0.05$) ALT (↓35%) and alkaline phosphatase (↓21%) were noted in the males at the end of the dosing period. Because these levels were decreased, as opposed to increased, the differences were not considered adverse. At the end of the recovery period, the following differences ($p<0.05$) were noted in the 10 mg/kg/day group: decreased triglyceride value in both sexes (↓31-57%); increased cholinesterase activity in the males (↑20%); elevated A/G ratio in the females (↑16%); and elevated chloride levels in the females (↑3%). Although the data were not provided, it was stated that the individual values for all of the above parameters were within the range of historical controls (control background data, 1995). With the exception of the significantly decreased ($p<0.05$) ALT and alkaline phosphatase, none of the above-mentioned differences were observed during the treatment period; therefore, these findings are considered incidental. All other statistically significant differences noted in clinical chemistry parameters were minor and/or unrelated to dose.

F. URINALYSIS: No treatment-related effects on urinalysis parameters were noted at any dose in either sex.

G. SACRIFICE AND PATHOLOGY

1. **Organ weight:** No treatment-related differences from controls were observed in absolute organ weights at any dose in either sex during the dosing or recovery periods. Relative (to body) kidney weight was increased ($p<0.05$) by 22% in the 10 mg/kg/day males at the end of the dosing period. Additionally, the following increases ($p<0.05$) in relative (to body) organ weights were observed in the 10 mg/kg/day females at the end of the dosing period: (i) kidney (↑28%); (ii) adrenals (↑32%); (iii) spleen (↑39%); (iv) brain (↑43%); (v) lungs (↑34%); and (vi) liver (↑43%). However, as no corroborative histopathological findings were observed, these decreases were attributed to the decreased body weights observed at this dose.

2. **Gross pathology:** At 10 mg/kg/day, treatment-related gross lesions were limited to slight to moderate atrophy of the biceps femoris in 2/5 females and slight to marked emaciation in all females at the end of the dosing period.

3. Microscopic pathology: In the 10 mg/kg/day females, increased incidence (# affected/5 vs. 0/5 controls) of muscle fiber atrophy was observed in the following tissues at the end of the dosing period (Table 5): (i) biceps femoris (2, slight); (ii) gastrocnemius (5, very slight to moderate); (iii) soleus muscle (5, very slight to slight); (iv) flexor digitorum longus (5, very slight to slight); and (v) anterior tibial muscle (5, very slight to slight). Very slight muscle fiber atrophy in the anterior tibial muscle was also observed in 2/5 males at 10 mg/kg/day (vs. 0/5 controls). All other microscopic differences from controls were considered incidental as they were commonly observed in rats, the severity was similar to controls, and/or they were unrelated to dose.

At the end of the recovery period, none of the males had any microscopic lesions. Very slight to slight muscle fiber atrophy was found in 2/5 females at 10 mg/kg/day in the gastrocnemius soleus muscle, flexor digitorum longus and anterior tibial muscle.

TABLE 5. Incidence (# affected/5) of muscle fiber atrophy in rats treated with copper pyrithione for 28 days. [a]

Observation		Dose (mg/kg/day)							
		0	0.6	2.5	10	0	0.6	2.5	10
		Males				Females			
Biceps femoris	Slight	0	0	0	0	0	0	0	2
Gastrocnemius	Total	0	0	0	0	0	0	0	5
	Very slight	0	0	0	0	0	0	0	1
	Slight	0	0	0	0	0	0	0	3
	Moderate	0	0	0	0	0	0	0	1
Soleus muscle	Total	0	0	0	0	0	0	0	5
	Very slight	0	0	0	0	0	0	0	1
	Slight	0	0	0	0	0	0	0	4
Flexor digitorum longus muscle	Total	0	0	0	0	0	0	0	5
	Very slight	0	0	0	0	0	0	0	2
	Slight	0	0	0	0	0	0	0	3
Anterior tibial muscle	Total	0	0	0	2	0	0	0	5
	Very slight	0	0	0	2	0	0	0	2
	Slight	0	0	0	0	0	0	0	3
Recovery period									
Gastrocnemius	Total	0	--	--	0	0	--	--	2
	Very slight	0	--	--	0	0	--	--	1
	Slight	0	--	--	0	0	--	--	1
Soleus muscle	Very slight	0	--	--	0	0	--	--	2
Flexor digitorum longus muscle	Very slight	0	--	--	0	0	--	--	2
Anterior tibial muscle	Very slight	0	--	--	0	0	--	--	2

[a] Data were extracted from Tables 11-2, 11-4, and 11-8 on pages 85, 87, and 91 of the study report.

-- Not evaluated

III. DISCUSSION AND CONCLUSIONS

A. REVIEWER'S COMMENTS: At 10 mg/kg/day, two females were sacrificed *in extremis* on Day 17 of the dosing period. These animals displayed the following clinical signs: emaciation from Day 14 of the dosing period onward; decreased spontaneous activity, piloerection, ataxic gait, and/or paralysis of the hind leg, urine stained abdomen, and reddish eye gum on Day 15 and thereafter; prone position on Day 16; and hypothermia, bradypnea, and lateral position on Day 17. Additionally, one 10 mg/kg/day female was found dead on Day 2 of the recovery period. This animal displayed the following clinical signs prior to death: emaciation from Day 17 of the dosing period onward; decreased spontaneous activity, ataxic gait and/or paralysis of the hind leg, urine stained abdomen, reddish eye gum, and a trace of reddish rhinorrhea from Day 18 onward; piloerection from Day 19 onward; prone position on Day 0 of the recovery period; and lateral position on Day 1 of the recovery period. While under observation using the Irwin method, an increase in passivity, decreases in reactivity, spontaneous activity, pain response, limb tone and grip strength, and loss of ipsilateral flexor reflex, abnormal hind limb posture and piloerection were observed on Days 20 and 27 of the dosing period. On Day 27, abnormal urination was also observed.

In the surviving 10 mg/kg/day females, emaciation was observed in all animals on Days 12 and 20 of the dosing period. Thereafter, piloerection decreased spontaneous activity, ataxic gait and/or paralysis of the hind leg were observed in 6 animals; reddish eye gum was noted in 3 animals; urine stained abdomen was noted in 2 animals; and prone position was noted in 1 animal. However, almost all of these findings disappeared or tended to recover by the end of the dosing period. Other than slight emaciation observed in three males on Days 21 to 28 or during Days 0-2 of the recovery period, no treatment-related clinical signs were noted in the males at any dose. During the FOB, incidence of the following observations were noted in the 10 mg/kg/day females on Day 20: (i) increased passivity; (ii) decreased reactivity; (iii) decreased spontaneous activity; (iv) decreased pain response; (v) abnormal hind limb posture; (vi) staggering gait; (vii) abnormal gait; (viii) decreased limb tone; (ix) decreased grip strength; (x) loss or decrease in ipsilateral flexor reflex; (xi) piloerection; and (xii) abnormal urination. On Day 27, the same FOB parameters still showed an effect of treatment in females at this dose; however, both incidence and severity were generally less than that observed during Day 20 observations. During the recovery period, a decrease in spontaneous activity and piloerection were still observed in 2 females and ataxic gait was noted in 1 female continuously from the dosing period. These abnormalities disappeared by Day 5 of the recovery period. Emaciation was also observed in 2 females but appeared to be recovering. Body weights were decreased in the males during Weeks 3 and 4 and in the females during Weeks 2-4. During the recovery period, body weights remained decreased in the males during Week 1 and in the females during Weeks 1-2. Body weight gains were decreased in the males during Weeks 2-4. The females at this dose actually lost weight during Weeks 2 and 3. Overall (Weeks 0-4) body weight gain was decreased in both sexes. During the recovery period, body weight gains were increased compared to controls in the males during Week 2 and in females during Weeks 1 and 2. Food consumption was decreased in the males during Weeks 3 and 4 and in the females during Weeks 2 and 3. Food

consumption was similar to controls in both sexes during the recovery period. Treatment-related gross lesions were limited to slight to moderate atrophy of the biceps femoris in 2/5 females and slight to marked emaciation in all females at the end of the dosing period. In females at this dose, increased incidence of very slight to moderate muscle fiber atrophy was observed in the following tissues: (i) biceps femoris; (ii) gastrocnemius; (iii) soleus muscle; (iv) flexor digitorum longus; and (v) anterior tibial muscle. Very slight muscle fiber atrophy in the anterior tibial muscle was also observed in the males at this dose (2/5 treated vs. 0/5 controls).

No treatment-related effects were observed at 0.6 or 2.5 mg/kg/day in either sex.

The LOAEL was 10 mg/kg/day based on decreased body weight, body weight gain, and food consumption in both sexes and on mortality, clinical signs of toxicity, and muscle fiber atrophy in the females. The NOAEL is 2.5 mg/kg/day.

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3050; OECD 407) for a subchronic oral toxicity study in the rat.

B. STUDY DEFICIENCIES: The following minor deficiencies were noted, but do not affect the conclusions or acceptability of this DER:

- Ophthalmoscopic evaluations were not performed.
- Several tissues were not collected and/or weighed during histopathological examinations.
- Details of the scoring scale for the Irwin method were not provided.
- Historical control data for the cited clinical chemistry data were not provided.

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